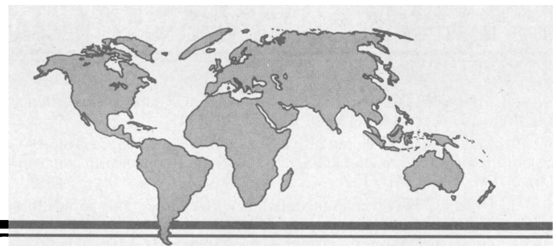

AIDS - A Global Perspective

Human Immunodeficiency Virus and Related Retroviruses

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This paper summarizes the current knowledge on the human immunodeficiency virus (HIV) and related retroviruses, describing basic characteristics of this new group of viruses such as morphologic and genetic structure, biological and cultural properties, virus growth characteristics, genetic variability and virus replication. The discovery of new human and simian retroviruses has prompted the World Health Organization (WHO) to convene a group of experts to establish criteria for their characterization. This will allow rapid identification of new variants that may arise and allow public health measures to be implemented accordingly. Different approaches are made to nomenclature in view of the evolution of knowledge about these viruses, and a system of nomenclature has been proposed by the WHO working group. This system, inspired by the one developed for the influenza viruses, is practical and descriptive, providing information on the origins of the organism and its type.

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Since it was endorsed by the International Committee on the Taxonomy of Viruses (ICTV), the name human immunodeficiency virus (HIV) has been adopted as the vernacular name, replacing the designations human T-lymphotropic virus type III (HTLV-III) and lymphadenopathy-associated virus (LAV).

The human retrovirus implicated as the agent causing the acquired immunodeficiency syndrome (AIDS) had been previously described by different authors under several designations, the first being that of the French group, which named it T-lymphotropic retrovirus,[1] followed by LAV,[2] HTLV-III,[3] immunodeficiency-associated virus,[4] AIDS-related virus (ARV)[5] and HTLV-III/LAV or LAV/HTLV-III. The last name was adopted by the World Health Organization (WHO) following the First International Conference on AIDS in Atlanta in 1985.

In May 1986, the committee recommended the name HIV to replace LAV/HTLV-III[6] in an attempt to unify the numerous previous designations of the virus, following the setting of criteria for establishing a uniform international nomenclature for viruses based on their taxonomy. According to the criteria, a retrovirus should be designated by a name having the following components: the species of the host from which the agent has been isolated, the major pathological property of the agent and the word "virus." This designation should be completed with a short name of the town (in parentheses) in which the isolation was made, in order to characterize it geographically, and an index allowing the identification of the individual viral organism.

By applying these criteria in naming HIV, the difference was established between this virus and HTLV-I and HTLV-II and any controversy avoided regarding priority of the discovery of the virus and emotive connections of the virus with the disease or its methods of transmission. However, as it was mentioned in a letter to *Science* by F. Brown, the president of ICTV, "Much still remains to be learned about the relationship of the human immunodeficiency virus with other retroviruses, and, therefore, designation of an international name would be premature."[6]

Therefore, it must be taken into account that the ICTV, as the official body of the Division of Virology of the International Union of Societies for Microbiology, has rules for virus nomenclature that should be followed before a name can be officially endorsed as international.[7] Thus, a new rule (No. 18) states that,

> The approval of the virus species proposed, species name and species type will be followed in two stages. In a first stage, a provisional approval can be obtained. These provisionally approved proposals will be published in an ICTV report. In a second stage, after a three-year interval, the proposals might obtain the final approval from the ICTV.

The apparent difficulties encountered and the care taken in taxonomy do not simply reflect questions of semantics, since the name of a virus contains, in a certain way, its essence. Thus, in a recent report from ICTV it is admitted that "The naming of virus species has lagged behind. It is first necessary to define what we mean by a virus species" and the proposal that "It should be defined as the smallest populations of viruses sharing a pool of genes maintained distinct from the gene pools of other viruses" has been vigorously debated.[8]

Classification of HIV

HIV is classified as belonging to the subfamily Lentivirinae according to its morphologic and morphogenetic characteristics, as it exhibits an electron dense cylindrical inner nucleoid. It is not tumorigenic or cell transforming in vitro, and its outer envelope glycoproteins show a progressive anti-

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ABBREVIATIONS USED IN TEXT

AGM = African green monkey
AIDS = acquired immunodeficiency syndrome
ARC = AIDS-related complex
ARV = AIDS-related virus
BLV = bovine leukemia virus
CAEV = caprine arthritis and encephalitis virus
EIAV = equine infectious anemia virus
HIV = human immunodeficiency virus
HTLV-III = human T-lymphotropic virus type III
IAV = immunodeficiency-associated virus
ICTV = International Committee on the Taxonomy of Viruses
LAV = lymphadenopathy-associated virus
mRNA = messenger RNA
SIV = simian immunodeficiency virus
STLV-III = simian T-lymphotropic virus type III
WHO = World Health Organization

genic drift. These characteristics coincide with those of the virus of visna disease in sheep and goats and of equine infectious anemia virus (EIAV) in horses. On the other hand, studies on the *pol* and *env* gene structure of different retroviruses also suggest the convenience of its classification among the lentiviruses.[9]

The group of lentiviruses could thus include HIV, EIAV, visna virus and caprine arthritis and encephalitis virus (CAEV), while other T-cell lymphotropic viruses such as HTLV-I and HTLV-II could be classified together with bovine leukemia virus (BLV) and possibly with simian T-cell lymphotropic virus (STLV) in a different group or family, the so-called E family or HTLV/BLV.

In Haase's 1986 review article on the pathogenesis of the lentivirus, the EIAV and AIDS viruses are also provisionally included in the lentivirus subfamily according to their pathogenic characteristics because they, too, cause slow infections and share other properties. These include cell fusion and other cytopathic effects in tissue culture, virion morphology, polypeptide composition, large envelope glycoproteins, shared antigenic determinants in the major structural protein *(gag)*, similar size and structure of their genomes and nucleotide and amino acid sequence homologies mainly confined to conserved regions of *gag-pol*.[10]

In that same year Gallo and his co-workers proposed a name and group that stressed the characteristic T-cell tropism of these viruses.[11] More recently, taking into account these and other characteristics, a new classification has been proposed. This would include HTLV-I, HTLV-II, HTLV-III, bovine leukemia virus, simian T-cell lymphotropic virus and some lentiviruses, such as visna. That new group has been named "type T retrovirus" by Haseltine and colleagues[12] on the basis that these retroviruses share a property that is not present in other retroviruses—a peculiar mechanism for replication, the transcriptional transactivation—which is the result of the expression of a unique gene called *x-lor* or *tat*. According to these authors, the most important differential characteristics of all these viruses could be their transactivating mechanism, which clearly sets them apart from all the other retroviruses. They favor the inclusion of all the viruses having that gene in a group—retrovirus type T—where all the different viruses could be identified by numbers: I, II, III and so forth.

The human immunodeficiency virus shares with the other two previously discovered human T-cell leukemia viruses the following properties:

- T-lymphocyte tropism, especially for T4.
- In vitro induction of giant, multinucleated cells.
- Mg^{++}-dependent reverse transcriptase.
- Three major "core" proteins present, with a relatively small but important p24.
- A p17 protein located in the NH_2 terminal end of the *gag* protein, as in HTLV-I and HTLV-II and opposite to other retroviruses that have a small phosphoprotein in between.
- Distant antigenic and genetic relationships.
- Presence of an extra gene, the *x-lor*.
- An open reading frame at the 3′ region whose transcript is 1.8 to 2.2 kb, messenger RNA (mRNA), coding for a p27 protein.
- The transactivating function, the long terminal repeat fragment and the presence of new kinds of regulatory elements for cells that are very likely functions of the p27 protein coded by the 2 kb RNA.
- A probable African origin.
- The presence of common antigenic epitopes such as the one located in p24 and others.

These same authors also point out the major differences in the genomes of these viruses, HTLV-I, HTLV-II and HTLV-III (HIV). Thus, it is mentioned that a close relationship between the viruses LAV and HTLV-I has been reported only by Barre-Sinoussi and co-workers[1] and Montagnier and colleagues,[2] discussing immunologic cross-reactivity or by Feorino and associates[13] reporting on the homology between nucleotide sequences.

In summary, the discovery of the human retroviruses HTLV-I and HTLV-II and subsequently of the retrovirus of AIDS has prompted a wealth of insight into these and other related viruses and brought a great amount of knowledge about their biological and biochemical characteristics.

All this new information might lead to a reclassification of the retroviruses. This need is important in itself, and it is an indicator of how the recent data available stress the different types of relationships existing between HIV and the adult T-cell leukemic retroviruses, HTLV-I and HTLV-II, on the one hand and some lentiviruses such as visna, CAEV, EAIV and BLV on the other.

In a recent meeting of the WHO Special Programme on AIDS Working Group on the characterization of HIV-related retroviruses, a system of nomenclature was proposed.[14] It was agreed that a practical and descriptive system that could provide information on the origins of the organism and its type would have considerable merit. Such a system could have the following components:

- The vernacular name of the virus: for example, HIV for organisms of human origin and simian immunodeficiency virus (SIV) for those of simian origin.
- An index for the serologic/genetic type of the virus (HIV-1, HIV-2, SIV-2) based on examination of the *env* glycoprotein components.
- In the case of viruses from nonhuman primates, the genus and species of the host from which the agent was isolated (*Macaca mulatta* for viruses from rhesus monkeys, for instance) should be specified.
- The country and laboratory in which the isolation was made.
- An index that allows identification of the individual virus—a strain number, for example.
- Year of isolation of the strain.

TABLE 1.—*HIV Seroprevalence in Spain—1986*

Group	No. Positive	%	No. Negative	%	Total
AIDS/ARC (suspect)	210	86.78	32	13.22	242
Intravenous drug abusers	331	63.05	194	36.95	525
Homosexual/bisexual men	29	12.83	197	87.17	226
Children born to seropositive mothers	46	86.79	7	13.21	51
Voluntary blood donors	43	0.05	86.571	99.95	86.614

AIDS = acquired immunodeficiency syndrome, ARC = AIDS-related complex, HIV = human immunodeficiency virus

- The country where the person or animal from whom the virus was isolated, if such information is available.

Examples of the use of this proposed nomenclature system for HIV/SIV strains are HIV-1/France, Pasteur/LAV BRU/83 (France); HIV-1/USA, NIH/HTLV-III B/83 (USA), HIV-I/Spain, Carlos III/MAJ/86 (Spain); HIV-2/France, Pasteur/LAV-2 MIR/85 (Guinea-Bissau).

HIV Characteristics

At the Cold Spring Harbor Workshop on AIDS held in 1983, R.C. Gallo proposed that AIDS was probably caused by a lymphotropic retrovirus, presumably related to the HTLVs.[15]

This hypothesis was based on epidemiologic evidence suggesting that the cause of AIDS was an infective agent and that it was transmitted by blood transfusions. It was thought it could be a virus because filtered blood products such as those used in the treatment of hemophilia patients were shown to transmit the disease. The target of that virus could be the helper/inducer T-lymphocytes subset (phenotype OKT4/Leu 3a$^+$), as their number was markedly decreased in AIDS patients. The only known infective agents with similar characteristics were the viruses HTLV-I and HTLV-II. On the other hand, it was known that another retrovirus, the feline leukemia virus was able to cause a disease in cats very similar to AIDS.

From that moment on, a systematic search was begun for a human retrovirus in lymphocytes. That search was possible because long-term growth of T cells had been previously achieved by Morgan and co-workers, who discovered the T-cell growth factor or interleukin 2[16] and also because very sensitive techniques for detecting the presence of retroviruses in cell cultures had been developed.[17-19]

In 1983 investigators from the Institute Pasteur in Paris published a report of the isolation of a retrovirus—then named T-cell human lymphotropic virus and that cross-reacted with HTLV-I—after some modifications were made in the cell culture protocols (addition of anti-α-interferon). As the virus was cytolytic for T cells, the amount of virus available was very small. It was studied by electron microscopy, but its association with AIDS could not yet be shown. After exchanging reagents for HTLV-I and HTLV-II with workers from the National Cancer Institute in the United States, the difference between the new virus and HTLV-I and HTLV-II could finally be established.

Several viruses were isolated: from patients with the lymphadenopathy syndrome, the lymphadenopathy-associated virus (LAV); and from patients with AIDS, the immunodeficiency-associated virus (IAV). However, the French organisms, in general, are usually referred to as LAV.

Montagnier and colleagues first detected the presence of specific antibodies in only 20% of AIDS patients, a result that could be attributed to the lack of an efficient system for viral replication.[2,20] In 1984 the group from the National Cancer Institute published several reports in which the HTLV-III virus and its association with AIDS were described.[3,21-23] The development of a permanent T4-lymphocyte cell line that was permissive to the virus but at the same time partially resistant to its cytopathic effect made this possible. This cell line could be chronically infected and cultured. Continuous production of the virus was possible and the cultures were enduring. Mass production of HTLV-III allowed its concentration and purification, and further biochemical and seroepidemiologic studies could then be performed.

The cell line H9, which was selected from among several cell lines that had been developed, was a derivative of a T-cell leukemic cell line. It could be kept in the laboratory by serial passages and could be cultured in the presence of fetal bovine serum without interleukin 2 or anti-α-interferon, thus facilitating its handling.

Later that year a third group, Levy and co-workers, isolated another virus from AIDS patients that was designated as AIDS-associated retrovirus (ARV).[5]

It is widely accepted that all these viruses are the etiologic agents of AIDS. This is supported by seroepidemiologic studies showing a clear association between the presence of antibodies and AIDS or the AIDS-related complex (ARC). A great number of studies have shown the presence of antibodies in 100% of AIDS patients and in almost all the ARC patients. On the other hand, the antibodies were undetectable in the controls. The presence of antibodies is an indicator of the rate of infection in the different populations affected.

Table 1 summarizes some of the serologic data from studies in our laboratory.[24] It can be seen that all the AIDS patients have antibodies. The groups with higher rates of infection are the intravenous drug abusers, children born to infected mothers and, with a considerably lower rate, homosexual or bisexual men. A high rate of infection in intravenous drug abusers has also been reported in other Mediterranean countries.

Morphology and Ultrastructure

Early pictures of HIV taken with an electron microscope by Montagnier's group showed that the virus shared with HTLV-I and HTLV-II not only some of their biochemical and biological characteristics but also their main morphologic features. The virus appeared as a spherical or quasi-spherical particle, with a 100-nm mean diameter, an outer envelope studded with spikes and an inner core containing a dense eccentric inner nucleoid. These features were one of the clues that helped to classify HIV in the Retroviridae family, as it appeared similar to the viruses of the "type C oncovirus group." The asymmetrical localization of the inner nucleoid or "central core" of these viruses was also a morphologic characteristic of HTLV-I and HTLV-II. More detailed morphologic studies, however, showed that this "central core" often displayed a tubular structure, a feature characteristic of the lentivirus subfamily (Figure 1).

Current knowledge of the HIV ultrastructure, supported by the work of Gelderblom and colleagues at the Robert Koch Institute in Berlin,[25] among others, shows that the virus's main morphologic features are the following:

- An outer envelope covered with knobs or spikes made up of the two envelope glycoproteins: gp 120, which is the outer spike component, and gp 41, which is attached to it and which sits in the viral lipidic membrane. It has been suggested that the distribution of gp 120 on the viral surface is similar to that of a soccer ball made of 12 pentagons and 20 hexagons stitched together to make a sphere. Gp 120 would be located at the corners of the hexagons, with an extra molecule at the center of each hexagon.
- An outer shell or core shell, which is composed of protein p17, arranged in an icosahedral structure and located at a very small distance from the outer envelope (unlike HTLV-I and HTLV-II).
- An inner nucleoid or central core made up of protein p 24 arranged in a helical pattern. In some pictures of the virus, this appears as a tubular structure, while in others this core is like a cone that is hollow, open at the narrow end (the top) and indented at the other end (the base).

As is characteristic for all the other retroviruses, the HIV structural components are assembled at the membrane of the cell it infects, in a process called "budding" (Figure 2).

It seems that the stages of this budding process are also different in HIV maturation from those described by Bernhard for type-C virus particles.[26] According to Gelderblom and associates, once the virus is released, shedding of gp 120 might occur, making it difficult for antibodies to stick to the free virus particles.[27] Further research on these subjects could also be of some help in classifying HIV in one subfamily or another.

The HIV envelope plays an important role in the specific cytopathic effect of HIV infection, syncytium formation and the like. The giant, multinucleated cells that appear in permissive cell cultures after infection are easy to detect by light microscopy and show a characteristic surface morphology when studied by scanning electron microscopy (Figure 3). These giant cells, which have been also observed in the brain by light microscopy and also appear in the blood of seropositive persons and AIDS patients (R. Nájera, MD, R. de Andres, MSc, I. Santa-Maria and M.I. Herrera, PhD, unpublished results, June 1986), could be a hallmark for this disease. However, a more precise characterization of infection by HIV should be achieved by means of specific immunolabeling of the cell. Immunofluorescence and immunoperoxidase techniques have been used, but the detection of the immune label by electron microscopy should prove to be more specific and sensitive. A technique that combines both the morphologic information and the rapid procedures of scanning electron microscopy with the precise antigen detection provided by the use of electron microscopy and immunolabeling with monoclonal antibodies has been recently applied to the detection of HIV antigens in infected cells by Herrera and co-workers.[28] A combination of high resolution scanning transmission electron microscopy and gold labeling techniques has been used to detect p 24, p 17 and gp 41 proteins in mononuclear cells from patients (Figure 4).

A wide variety of applications of this method to the study of AIDS, such as the early diagnosis, monitoring patients during antiviral therapy and others, can be expected.

Biological Properties

In vitro infection of human lymphocytes by HIV is characterized by a burst of virus production occurring one to three

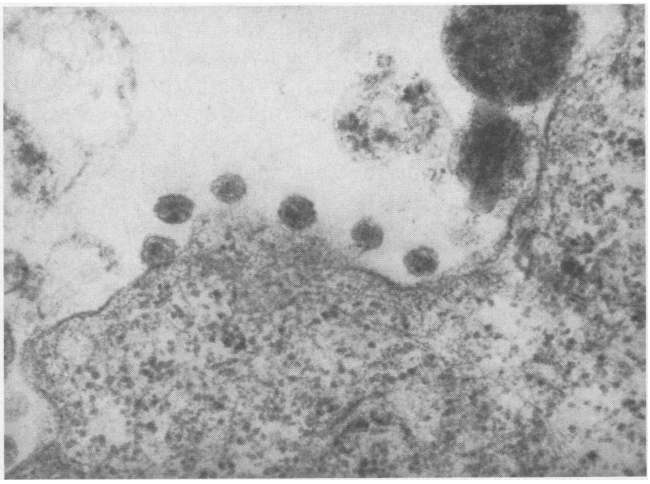

Figure 1.—The transmission electron micrograph shows human immunodeficiency virus particles invading a cell (× 72,000).

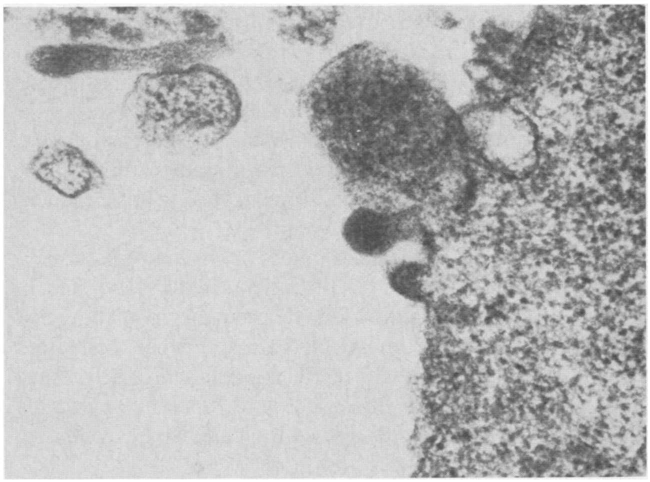

Figure 2.—The transmission electron microgram shows human immunodeficiency virus budding from a cell (× 155,000).

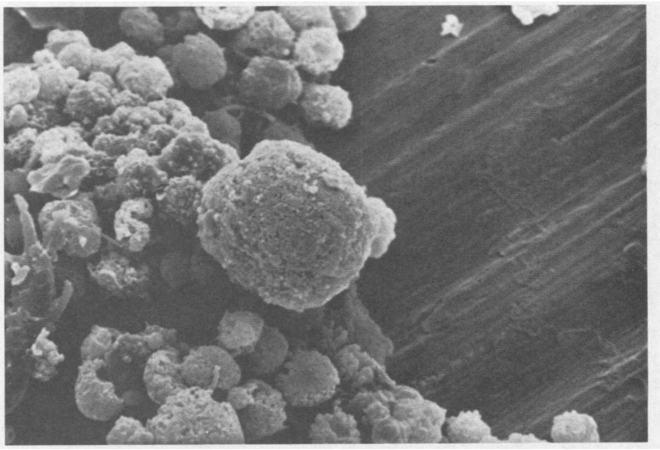

Figure 3.—The scanning electron micrograph shows a group of human immunodeficiency virus-infected cells, with a "giant" cell in the center (× 1,800).

weeks following infection. Within the heterogeneous population of T cells, T4 lymphocytes (phenotype OKT 4/Leu 3 a$^+$) are apparently infected, but it has been observed that other cell types, such as Epstein-Barr virus-transformed B lymphocytes, Langerhans' cells and macrophages, can also be infected. It has been reported recently that the major type of cell that can be productively infected with HIV in brain tissue is the mononuclear phagocyte, as suggested by the detection of budding virus particles and the long-term production of infectious virus in the primary cultures.[29] Virus particles have also been detected in astrocytes and microglial cells, however, but not in neurons, oligodendrocytes, pericytes or endothelial cells, though some damage to endothelial cells has been reported in children with AIDS having unusual basal ganglia calcification.[30]

Virus Isolation

The human immunodeficiency virus is generally cultured from specimens of peripheral blood from infected persons. The virus frequently has been identified in semen, lymph nodes and brain tissue and very seldom in saliva, sweat or bronchial exudates.

Mononuclear cells from peripheral blood are obtained after layering a specimen of heparinized venous blood over a Ficoll-Hypaque density gradient and centrifuging the specimen at 750 g for 20 minutes. The mononuelear cells are then collected, activated with phytohemagglutinin for two to three days and cultured in a medium supplemented with interleukin 2. The virus can be detected either with reverse transcriptase assays of the tissue culture fluid, by immunofluorescence or by electron microscopy for the presence of antigens in cells. Once isolated, the virus can be transmitted to other cells, mostly to umbilical cord blood lymphocytes or to permanent cell lines of T4 or macrophage origin.

The virus can be identified in 20% of symptomless seropositive individuals, in 50% of patients with AIDS and in 80% of the patients with ARC. The smaller percentage of organisms obtained from AIDS patients, when compared with the number of them from ARC patients at an earlier stage of the disease, could be explained by the specific cytopathic effect of this virus, as viral replication can also be cytotoxic, depending on the cell type.

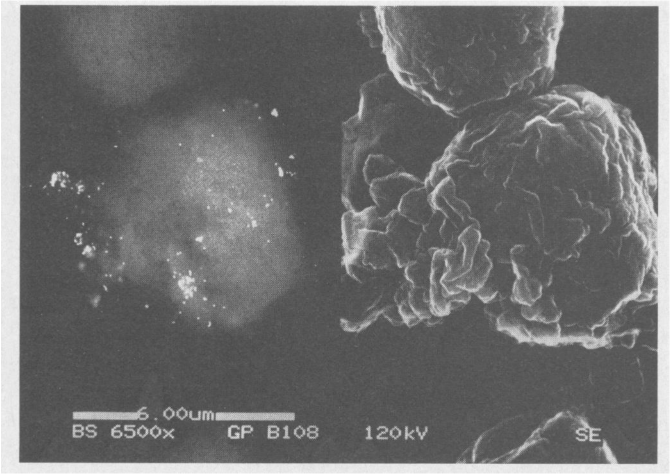

Figure 4.—The paired scanning/transmission electron micrograph of peripheral blood mononuclear cells from a patient seropositive for human immunodeficiency virus antibodies shows alterations in surface morphology (secondary electrons image) and gold-labeled antigens (backscattered electrons image) (× 6,500).

Cytopathic Effect

One effect of the infection of lymphocytes by HIV is the formation of multinucleated giant cells or syncytia that die out rapidly. Syncytial formation appears to result from the action of two antigens that are the products of the *env* gene of HIV, the proteins gp 160 and gp 120; gp 160 is considered to be a precursor of gp 120, the outer spike antigen. Infected cells express this antigen in their surface. As a result, they have a great avidity for binding to cells expressing the T4 phenotype (or permissive cells) and can serve to nucleate the formation of syncytia with them via a fusion reaction. This effect may help to explain the marked depletion of T4 lymphocytes observed in AIDS patients despite the small percentage of peripheral blood lymphocytes actively producing viral messages at any given time.[31]

The cytopathic effect can be clearly seen by light microscopy in fresh preparations, and for this reason it is used as an indicator of viral growth.

Genetic and Antigenic Components

The genome of HIV is similar to those of the other retroviruses, with three major structural genes, *gag, pol* and *env,* that are flanked by sequences repeated on both ends of the genome (5′ and 3′ terminals).

The *gag* gene codes for the group-specific proteins or group antigens. The *pol* gene codes for the polymerases, the enzymes required for viral replication, a reverse transcriptase and an endonuclease. The first one, the reverse transcriptase, which gives name to the retrovirus family, is an enzyme that transcribes the RNA genome into a DNA provirus, which then becomes integrated into the host chromosomal DNA. The *env* gene codes for the external envelope proteins of the virus that serve to bind the virus to the receptor of the permissive cells and that play a role in the neutralization of the virus.

However, the genome of HIV is much more complex than that of the other retroviruses, as it consists of eight genes encoding different proteins. The genome coding potential and protein processing in HIV are summarized in Table 2.

Genetic Variability

Since the studies on HIV began, its great variability has become apparent. LAV and HTLV-III differ in 1.5% of their sequences. However, the variations among other viruses are even greater: for example, ARV and RF-II show a 10% variation.

The recently isolated virus LAV-2[32] differs from the previous one (LAV-1) to such a great extent that it supports its classification as another subgroup. LAV-2 was isolated from two patients without antibodies against LAV-1. The genomic differences between LAV-1 and LAV-2 are estimated to be 30%. People infected with LAV-1 do not have antibodies against LAV-2 envelope proteins; however, serum from LAV-2-infected patients cross-reacts with LAV-1 core proteins.

Another virus, HTLV-IV, isolated from three healthy Senegalese, shows antigenic cross-reactivity and similar characteristics to a simian virus called STLV-III African green monkey (AGM) that was isolated from rhesus monkeys that were kept in captivity and had an AIDS-like illness. Further studies showed that this virus had infected 50% of asymptomatic wild African green monkeys. The Swedish group of Biberfeld has also isolated a virus (SBL-6669) serologically

TABLE 2.—*Human Immunodeficiency Virus Type 1 Genes and Gene Products*

Coding Gene	*Protein*
gag	p 24(p 25)*, p 17(p 18)*, p 15(p 13)* (group antigen) p 53-55 (precursor)
pol	p 66/p 51(p 68/p 53)* (reverse transcriptase) p 32-34 (endonuclease)
sor (*Q*)†	p 23
tat	p 14 (transactivator)
env	gp 120(gp 110)* gp 41 (envelope) gp 160 (precursor)
3'orf (*F*)†	p 27
trs (*art*)	p 16 (antirepressor)
R	—

*Alternative molecular weight values.
†Alternative gene designation.

related to the last two.[33] All of them share similarities in their *env* glycoprotein components.

The only difference existing between LAV-2 and HTLV-IV is that LAV-2 has been isolated from patients, while HTLV-IV has been found in healthy individuals. On the other hand, though both of them can infect permissive cells, the effect of LAV-2 is cytolytic, causing the cells to die, while HTLV-IV is able to infect cells but not kill them. The serologic data suggest that HTLV-IV shares antigenic epitopes with STLV-III AGM but not with the etiologic agent of AIDS—LAV/HTLV-III.[34]

Recently, Hahn and co-workers studied the genetic variation of the virus over time in the same patient.[35] The changes detected in the viruses obtained over a two-year period extended throughout the viral genomes and consisted of clustered and isolated nucleotide point mutations as well as short deletions or insertions. A comparative analysis indicated that the viruses had evolved from a common progenitor virus.

The rate of evolution was estimated to be at least 10^{-3} nucleotide substitutions per site per year for the *env* gene and 10^{-4} for the *gag* gene, in agreement with the data on variability found for other RNA viruses (one million-fold greater than for most DNA genomes).

This evolutive model may suggest that once infected with one AIDS virus, individuals are protected from infection with other genotypically different viruses. Some type of interference mechanism appears to prevent simultaneous infection, even in cases such as homosexual men who reported having more than a thousand different sexual partners.

Variations in the *env* gene are especially important, as this gene codes for the external proteins to which the specific antibodies should attach. It appears that some type of immunologic pressure is exerted, inducing the mutant selection.

The distribution of nucleotide substitutions and deletions or insertions is not uniform throughout the *env* gene. Instead, such changes are clustered in the exterior portion of the envelope glycoproteins and coincide with the regions that, based in their secondary structure, glycosylation pattern and hydrophilicity, represent predicted antigenic sites.[36]

This great genetic variation, with its corresponding effect on antigenicity, is perhaps the greatest problem encountered in developing a vaccine, a problem that is similar to that of the influenza viruses. A better understanding of the mechanism used by the virus to kill the T cell has been achieved, however, and it has been possible to develop laboratory variants whereby, through a deletion in the *env* gene, viruses with a reduced killing potential have been obtained.[37] These viruses could be used to develop a vaccine because the two functions, replication and cytotoxicity, would be separated in them. It appears that the latter is associated with a piece of the *env* gene product at its carboxyterminal.

HIV Replication

The human immunodeficiency virus is attached to the cell by an interaction of the envelope glycoproteins with the cellular receptor. Fusion of the viral envelope with the cell membrane occurs, and the inner core of the virus enters the infected cell. At this time, under the control of the reverse transcriptase, decapsidation takes place and the nucleic acid is released. Once the RNA is copied into a DNA provirus, this becomes integrated in the cellular DNA but, in contrast with other retroviruses, nonintegrated DNA may accumulate in the cytoplasm of chronically infected cells.

Integration of the DNA transcript as a provirus is essential for virus replication. From its integration site in the host chromosomal DNA, the virus completes its replication cycle by directing the transcription into the messenger RNAs. Some of them have been already identified: a 4.2-kb RNA could be the messenger for synthesizing *env* proteins and two 2.2- and 2.0-kb strands of RNA could be used to synthesize the products of the *tat* and *3'orf* genes.

However, HIV shows a new and complex pattern of replication. The virus is able to stimulate its own replication in the infected cell. This autostimulation would be mediated by a bipartite gene, the *tat* gene, together with another gene called *art* (also bipartite). This would express an antirepressor of translation, activating the functions of the *gag* and *env* genes and, in consequence, regulating the viral replication.

The virus remains in an infected person for a lifetime as a part of that person's genetic material integrated in the cellular DNA as a provirus. It may also be found in the cytoplasm as extrachromosomal DNA. Under stimulation, the lymphocyte replicates its DNA, and, simultaneously, the viral DNA is also replieated and infectious virus is produced.

Integrated viral DNA has been detected in tissue specimens from patients with AIDS or ARC. Viral expression has been frequently demonstrated in the lymph nodes, spleen, brain and peripheral blood from patients.

REFERENCES

1. Barre-Sinoussi F, Cherman JC, Rey F, et al: Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immunodeficiency syndrome (AIDS). Science 1983; 220:868-870

2. Montagnier L, Gruest J, Chamaret S, et al: Adaptation of lymphadenopathy associated virus (LAV) to replication in EBV-transformed B-lymphoblastoid cell lines. Science 1984; 225:63-66

3. Popovic M, Sarngadharan MG, Read E, et al: Detection, isolation and continuous production of cytopathic retrovirus (HTLV-III) from patients with AIDS and pre-AIDS. Science 1984; 224:497-500

4. Vilmer F, Barre-Sinoussi F, Rouzioux C, et al: Isolation of a new lymphotropic retrovirus from two siblings with haemophilia B, one with AIDS. Lancet 1984; 1:753-757

5. Levy JA, Hoffman AD, Kramer SM, et al: Isolation of lymphadenopathic retroviruses from San Francisco patients with AIDS. Science 1984; 225:840-842

6. Brown F: Human immunodeficiency virus. Science 1986; 232:1486

7. Matthews REF: Classification and nomenclature of viruses—Fourth report of the International Committee on Taxonomy of Viruses. Intervirology 1982; 17:1-3

8. Brown F: The classification and nomenclature of viruses: Summary of results of meetings of the International Committee on Taxonomy of Viruses in Sendai, September 1984. Intervirology 1986; 25:141-143

9. Yasunaga T, Sagata N, Ikawa Y: Protease gene structure and *env* gene variability of the AIDS virus. FEBS Lett 1986; 199(2):145-150

10. Haase AT: Pathogenesis of lentivirus infections. Nature 1986; 322:130-136

11. Gallo RC, Sarngadharan MG, Popovic M, et al: HTLV-III and the etiology of AIDS. Prog Allergy 1986; 37:1-45

12. Haseltine WA, cited in Gallo RC, Sarngadharan MG, Popovic M, et al: HTLV-III and the etiology of AIDS. Prog Allergy 1986; 37:1-45

13. Feorino PM, Kalyanamaran VS, Haverkos VS, et al: Lymphadenopathy associated virus infection of a blood donor-recipient pair with acquired immunodeficiency syndrome. Science 1984; 225:69-72

14. World Health Organization Special Programme on AIDS Working Group on Characterization of HIV-Related Retrovirus. Madrid, June 15-16, 1987 (memo)

15. Gallo RC, Essex M, Gross L: Human T-Cell Leukemia-Lymphoma Virus: The Family of Human T-Lymphotropic Retroviruses—Their Role in Malignancies and Association with AIDS. New York, Cold Spring Harbor Press, 1984

16. Morgan DA, Ruscetti FW, Gallo RC: Selective "in vitro" growth of T-lymphocytes from normal human bone marrow. Science 1976; 193:1007-1008

17. Baltimore D: RNA-dependent DNA polymerase in virions of RNA tumour viruses. Nature 1970; 226:1209-1211

18. Temin HM, Mizutani S: RNA-dependent DNA polymerase in virions of Rous sarcoma virus. Nature 1970; 226:1211-1213

19. Sarngadharan MG, Allaudeen HS, Gallo RC: Reverse transcriptase of RNA tumor viruses and animal cells. Methods Cancer Res 1976; 12:3

20. Montagnier L, Chermann JC, Barre-Sinoussi F, et al: A new human T-lymphotropic retrovirus: Characterization and possible role in lymphadenopathy and acquired immunodeficiency syndrome, *In* Gallo RC, Essex M, Gross L: Human T-Cell Leukemia-Lymphoma Virus: The Family of Human T-Lymphotropic Retroviruses: Their Role in Malignancies and Association with AIDS. New York, Cold Spring Harbor Press, 1984, pp 363-379

21. Schupbach J, Sarngadharan MG, Gallo RC: Antigens on HTLV-III infected cells recognized by leukemia and AIDS sera are related to HTLV viral glycoproteins. Science 1984; 224:607-610

22. Gallo RC, Salahuddin SZ, Popovic M, et al: Frequent detection and isolation of cytopathic retrovirus (HTLV-III) from patients with AIDS. Science 1984; 224:500-503

23. Sarngadharan MG, Popovic M, Bruch L, et al: Antibodies reactive with human T-lymphotropic retrovirus (HTLV-III) in the serum of patients with AIDS. Science 1984; 224:506-508

24. Nájera R: El Virus del SIDA, chap 1, SIDA: Un problema de salud Pública. Madrid, Ediciones Díaz de Santos, 1987, pp 1-48

25. Gelderblom H, Ozel M, Pauli G: T-Zell-spezifische Retrovir des Menschen: Vergleichende morphologische Klassifizierug und mógliche funktionelle Aspekte. Bundesgesundhbl 1984; 28(6): 161-172

26. Bernhard W: Oncornaviruses: Type A and C virus particles in murine and other mammalian leukemias and sarcomas, *In* Dalton AJ, Haguenau F (Eds) Ultrastructure of Animal Viruses and Bacteriophages: An Atlas. New York, Academic Press, 1973

27. Gelderblom HR, Reupke H, Pauli G: Loss of envelope antigens of HTLV-III virus and pathogenesis of AIDS. Lancet 1985; 2:1016-1017

28. Herrera MI, Santa-Maria I, de Andres R, et al: Localization of human immunodeficiency virus antigens in infected cells by scanning/transmission-immunogold techniques. Ultrastructural Pathology, in press

29. Gartner S, Markovits P, Markovitz DM, et al: Virus isolation from and identification of HTLV-III/LAV-producing cell in brain tissue from a patient with AIDS. JAMA 1986; 256:2365-2371

30. Barnes DM: Brain function decline in children with AIDS (News). Science 1986; 232:1196

31. Sodroski J, Goh WC, Rosen C, et al: Role of the HTLV-III/LAV envelope in syncytium formation and cytopathicity. Nature 1986; 322:470-474

32. Clapham PR, Weber JB, Montagnier L, et al: Common and variable neutralization antigens of HTLV-1 and HTLV-2—Proceedings of the 3rd International Conference on AIDS, Washington, DC, June 1987

33. Norrby E, Biberfeld G, Albert J, et al: Significance of variation between human immunodeficiency (HIV) isolates for serology and vaccine development—Proceedings of the 3rd International Conference on AIDS, Washington, DC, June 1987

34. Essex M, Kanki P, Marlink R, et al: Human and simian T-lymphotropic retroviruses: Serologic identification and vaccine development—Proceedings of the 3rd International Conference on AIDS, Washington, DC, June 1987

35. Hahn BH, Shaw GM, Taylor ME, et al: Genetic variation in HTLV-III/LAV over time in patients with AIDS or at risk of AIDS. Science 1986; 232:1548-1553

36. Hahn BH, Shaw GM, Wong-Staal F, et al: Genomic variation of HTLV-III/LAV, the retrovirus of AIDS, *In* Fields B, Martin MA, Kamely D (Eds): Genetically Altered Viruses and the Environment. New York, Cold Spring Harbor Press, 1985

37. Fisher AG, Ratner L, Mitsuya H, et al: Infectious mutants of HTLV-III with changes in the 3′ region and markedly reduced cytopathic effects. Science 1986; 233:655-659